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Molecular Loci for Potential Drug Toxicity in Ovaries

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Ovarian follicular development is dependent on the actions and interactions of the pituitary gonadotropins, FSH and LH, and the ovarian steroid hormone, estradiol. Agents which might block the effects of these hormones would increase follicular atresia and reduce fertility. In addition, any substance toxic to the oocyte and its normal pattern of growth and meiosis would lead to reduced numbers of oocytes and follicles and impaired fertility. Autoimmune diseases may be one major cause of premature ovarian failure, and such diseases might be triggered by toxic external stimuli.

The ovarian follicle consists of three cell types: the germ cell (or oocyte), granulosa cells and thecal endocrine cells. The growth, maturation and differentiation of each cell type is required for successful ovulation of a fertilizable ovum and formation of a corpus luteum. Implantation of a fertilized egg subsequently requires the maintenance of a functional corpus luteum.

Interruption of the events leading to follicular ovulation and luteinization or successful maturation of oocytes can occur at many stages. The hypothalamic–hypophyseal–gonadal feedback system itself selects only a few follicles for ovulation. For example, the human ovary contains approximately two million eggs at birth. However, during the reproductively active years of women only 480 eggs ever ovulate (*1*). In fact, 80% of the oocytes and follicles contained in the ovary at birth have been lost (degenerate) before the first ovulation ever occurs! With so many follicles degenerating or becoming atretic and so few being selected, it is important to know how this stringent biological selection process works and which steps might be most sensitive to toxic external stimuli.

Three major processes which occur during the life span of a follicle, and which might be susceptible to toxic substances include: mitosis of oogonia and granulosa cells occurring at specific stages of follicular growth; meiosis of oogonia to form oocytes; and differentiation of granulosa cells and theca cells permitting them to respond to the LH surge and ovulate. These three processes occur during the life cycle of a follicle as described below (Fig. 1).

Follicle Formation

Formation of follicles occurs during fetal life (in the human) or immediately after birth (as in the rat). As germ cells invade the undifferentiated ovary, they are undergoing mitosis and populate this tissue as if it were a nest. Subsequently the germ cells enter the prophase of meiosis and other cells within the ovary begin to surround each oocyte. The ovarian cells which are in direct contact with the egg are called granulosa cells. In the "resting" or primordial (nongrowing) follicles, these granulosa cells appear flat. Follicles can remain embedded in the ovary like this for as long as 55 years! Agents which might inhibit the migratory behavior of oogonia as they move from the yolk sac via the blood stream to ovary would prevent fertility. Such agents would have to pass the fetal-placental blood barrier. Agents, such as irradiation, which damage cells in

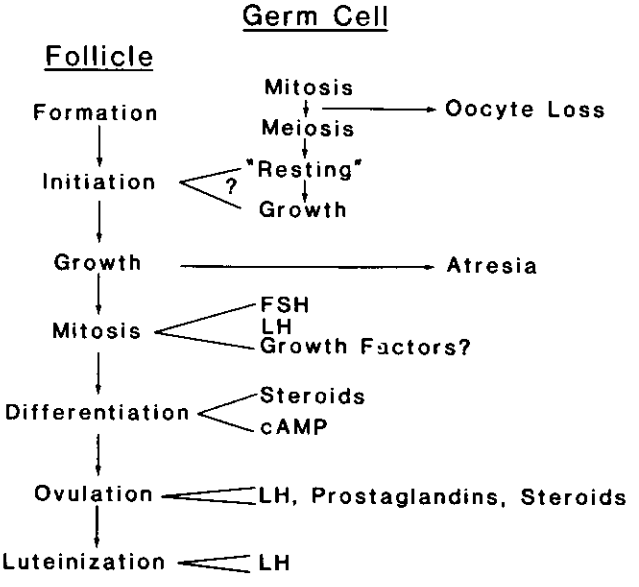

FIGURE 1. Formation and maturation of an ovarian follicle: cellular and hormonal events.

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meiosis could reduce fertility by causing meiotic germ cells to die (*1*). Because many of the germ cells are dying at this stage "normally," if we knew the reasons for this normal loss we might be in a position to reverse it. Lastly, oocytes which remain in the resting phase of meiosis for 50 years or more are often associated with increased chromosomal abnormalities (*2*). The causes are unknown.

Initiation of Follicle Growth

Initiation of follicular growth occurs continuously until menopause. That is, each day from birth to menopause a certain number of follicles begin to grow. Growth initiation is characterized by three events: the oocyte begins to enlarge; the granulosa cells change from their flattened form to a rounded configuration; and the complex protein/carbohydrate extracellular matrix between the granulosa cells and oocytes, called the zona pellucida, appears. This first stage of growth typifies the class of follicles known as primary follicles. The hormones or factors which trigger the initiation of growth of any given follicle on a given day are as yet unknown. The pituitary hormones, FSH and LH, appear not to be involved in this first step. For lack of a better explanation, some biologists have said this process occurs by chance. However, as more is learned about the biology and molecular biology of development in other systems, the secret to this first step of follicular growth may be unveiled. When this secret is uncovered, it should be possible to inhibit or speed up this process for the specific needs of fertility control.

Follicular Growth

Growth of the ovarian follicle is associated with five events: the oocyte continues to grow; the number of layers of granulosa cells increases; the basal lamina, an extracellular matrix external to the outer layer of granulosa cells is formed; the endocrine thecal cells organize around the basal lamina; and the fluid filled cavity, called an antrum, appears.

The multiple layers of granulosa cells within the follicle are formed by the progressive increase in the number of granulosa cells within the follicle. The most elegant demonstration of their remarkable rate of cell division has been provided by the studies and photographs of Hirshfield (*3*). This mitotic activity is maintained throughout follicular growth and ceases only when either luteinization or atresia occurs.

Agents which inhibit cell division would inhibit granulosa cell growth and cause an increase in follicular atresia. Because the growth of the ovarian follicle during this time is dependent on FSH and LH, any agent blocking either the synthesis or action of the gonadotropins would cause an increase in atresia and a decrease in fertility. Some agents which have been shown to increase atresia are GnRH (*4*), EGF, and high concentrations of androgens (*5*). Each of these has been shown to impair the response of granulosa cells to FSH, primarily by altering the synthesis or intracellular action of cyclic AMP (cAMP). The stages of follicular growth which are most sensitive to these inhibitors remains to be clearly defined.

Follicular Differentiation

Differentiation is the hallmark of those few ovarian follicles which successfully develop to a stage capable of undergoing ovulation. Remember this select group includes only 480 out of the two million follicles contained in the human ovary at birth. What regulates this stringent biological selection process? If all follicles within the ovary are exposed to the same cyclic changes in FSH and LH, why do some ovulate whereas others do not? Why do some follicles become atretic whereas others continue to grow?

The key to the development of a follicle which succeeds in ovulating lies in the ability of that follicle to acquire new functional capabilities in response to the same signals, FSH and LH. Yet, one might ask how can the same hormone (FSH) acting on granulosa cells via the same receptor (R_{FSH}) cause different effects depending on whether the granulosa cells are from small follicles or large follicles? Likewise, how can LH acting on theca cells elicit different effects in small follicles versus large follicles? These are not only intriguing questions, but also appear to be the secret of how the ovary maintains continuous growth of small follicles and at the same time selects specific follicles for ovulation (*5*).

To summarize the results of many experiments we can say that the tonic secretion of FSH and LH maintains the growth of follicles to the small antral stage and a subtle increase in LH (or increased responsiveness to LH) is required for the final stages of follicular growth. The increase in LH stimulates the conversion of progesterone to androstenedione by the 17α-hydroxylase/$C_{17\text{-}20}$ lyase enzyme in theca cells. The androgens are then converted to estradiol in granulosa cells. Estradiol acting with FSH (or cAMP) is obligatory for regulating the differentiation of granulosa cells including the increased cellular content of LH receptors, FSH receptors, aromatase activity, cholesterol side chain cleavage, cytochrome P-450 ($P\text{-}450_{CSCC}$) and prostaglandin synthetase (*5,6*). The last of these regulates the synthesis of prostaglandins which are increased by the LH surge and are then obligatory for ovulation to occur.

The essence of this brief overview is that the differentiation of theca cells (increased androgen synthesis) determines which follicles will gain the substrates necessary for estradiol synthesis. Only those follicles capable of producing estradiol become preovulatory follicles (Fig. 2) (*5,6*). Thus, any agent which inhibits either theca cell function (ability to synthesize androgens) or granulosa cells function (synthesis and action of estradiol) will cause atresia. Furthermore, because both LH and FSH act via cAMP, agents which alter gonadotropin receptor content or the functional cou-

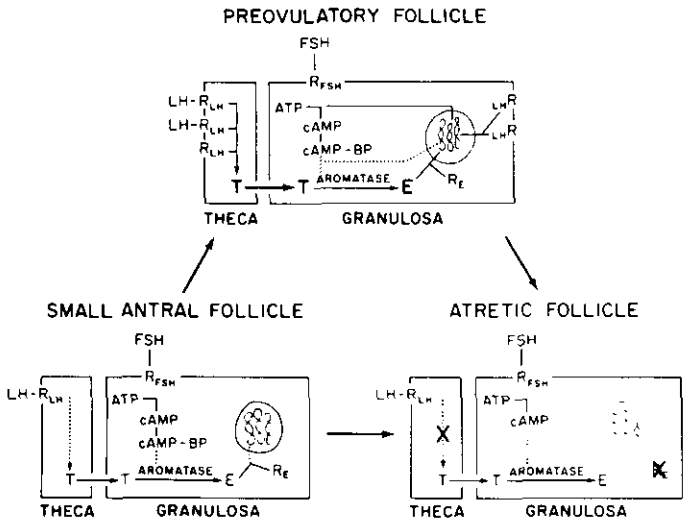

FIGURE 2. Roles of FSH, LH, and estradiol on follicular cell function.

pling of the receptor to adenylate cyclase will also cause atresia and lowered fertility.

Premature ovarian failure can also be linked to the immune system. For example, fetal thymectomy in monkeys (*7*) and mice (*8,9*) leads to abnormal ovarian differentiation in adult animals. Exposure of animals to the glycoprotein components of the zona pellucida leads to the synthesis of antibodies and the dramatic loss of oocytes from the ovary (*10*). Thus, premature menopause could be linked to an autoimmune disease in which antibodies to the zona pellucida or other structures of the follicle could result in the loss of follicles.

Atresia as it occurs naturally is a poorly understood process but plays a key role in fertility (*11*). Atresia may result from gonadotropin concentrations which are too low or may be stimulated by an endogenous antagonist. As more and more peptides within the ovary are characterized it is possible that a specific atretogenic factor will be identified.

In summary, external agents may interfere with fertility at the ovarian level by acting at many sites to: (1) decrease the concentrations of gonadotropins, (2) destroy oocytes, (3) cause autoimmune mechanisms to operate, (4) increase antigonadal peptides, (5) inhibit the actions of LH and FSH (cAMP), (6) inhibit the action of estradiol, (7) prevent luteinization, (8) increase synthesis luteolytic factors, and (9) alter gene transcription and prevent differentiation.

The tools to measure many of the functional aspects of granulosa and theca cell differentiation (such as hormone receptors) are now available. Recombinant DNA technology is also going to provide new and sensitive tools with which to identify how various agents alter ovarian cell function (*12–15*). These tools, applied to the toxicology of the ovary, could provide important new insight into the control of follicular and luteal cell function.

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